ERDC TN-DOER-C22
September 2001

Toxicological and Bioaccumulation Testing of Dredged Material in Confined Disposal Facilities Using Plants and Worms

PURPOSE: Contaminants in dredged material of confined disposal facilities (CDFs) can move from substrates into food webs because of their contact with CDF-colonizing or -inhabiting plants and animals, and as such cause unacceptable risks outside the CDF. The primary goal of this technical note is to provide guidance on how to determine exposure-based effects on index and other species, and how to relate these effects to the fates of contaminants in food chains. The toxicity of dredged material from CDFs and the bioaccumulation of contaminants from this material were determined in test species. The plants *Cyperus esculentus* (yellow nutsedge) and *Cynodon dactylon* (common bermudagrass) and the invertebrates *Eisenia fetidan* (earthworm) and *Enchytraeus crypticus* (enchytraeid worm) were used as test organisms. At a later stage, exposure-based effects evaluation and interpretive guidance will be provided for effective management of contaminants to minimize unacceptable risks in CDFs and for beneficial use of dredged material projects.

BACKGROUND: Placement of dredged material in CDFs and its removal from CDFs for beneficial use require assessment of environmental risk. To this end the Decision-Making Framework and the U.S. Army Corps of Engineers (USACE)/U.S. Environmental Protection Agency (USEPA) Technical Framework may require exposure and effects assessments of relevant contaminant pathways prior to dredging to evaluate impacts on plants and animals in cases where terrestrial placement is selected as a disposal alternative, and there is a reason to believe that the dredged material is unacceptably contaminated. Currently no specific guidelines for contaminant residues in plants and animals exist. Moreover, risk assessment based on assessments performed on index species may not necessarily give an accurate indication of the fate and effects of the contaminants because the index species may have little relevance to those species inhabiting CDFs or species found where the dredged material can be put to a beneficial use.

INTRODUCTION: USACE annually manages about 300 million cu m of dredged material. The traditional placement of 5 to 10 percent of material, contaminated enough to require special handling, into CDFs is rapidly becoming problematic because most CDFs are at or approaching their design capacity. USACE Districts are working together with Federal and State Agencies to develop tools that help integrate topical quantitative information to yield quantifiable estimates of risk posed by dredged materials including uncertainty (Moore, Bridges and Cura 1998). The use of effects-based testing and risk assessment is intended to supplement the analytical options currently available to dredged material managers by building on the existing technical framework (USEPA/USACE 1992) and the existing tiered approaches (USEPA/USACE 1991, 1998). However, even after these tools have become available and linked to databases regarding various aspects of ecotoxicology of dredged material, the data pertinent to upland CDFs remain extremely limited because of the unique characteristics of these materials. Standardized and dependable assessments pertaining to relevant biota are needed for credible risk management.

INITIAL SURVEY OF CDFs: A limited survey of USACE Districts was conducted to obtain the following information on upland CDFs within the United States collected on the sediment before dredging:

- Contaminant types and levels.
- Bioassays of dredged materials.
- Freshwater or marine origin.

and after placement into the CDF:

- Chemical behavior of contaminants.
- Survey of biota.
- Bioassays of dredged materials.
- Availability of dredged material for bench- and field-scale testing.
- Easy accessibility of the CDF.

This initial survey focused on freshwater dredged material. The goal was to identify one site to serve as an example for testing of inorganics and one other site to serve as an example for testing of organics. Based on the information collected, three sites in the Great Lakes area with known predredging contaminant types and levels were initially selected for verification of chemical composition, evaluation of nutrient levels, and preliminary survey of biota: Monroe, MI, Manitowoc, WI, and Bayport, WI (Figure 1). The three sites were visited on 5 and 6 June 2000, their locations were recorded (in universal transverse Mercator coordinates), landscape/ vegetation characteristics were summarized, dredged material samples were collected and shipped on ice to the respective laboratories for chemical/ physical analysis, and other dredged material samples were collected for macroinvertebrate survey purposes.

Figure 1. Upland CDFs initially explored for site selection

Landscape and Vegetation Characteristics

Monroe, MI. This CDF, located within a state park along Lake Erie, is typical for many CDFs in the Great Lakes area that have been in existence for several years or more. It is composed of a large ponded area on the discharge side transitioning upwards into mud flats and wetland areas. The site is still in use for the disposal of dredged material. The upland area was occupied largely by trees, among which willow and cottonwood predominated. The site showed signs of recent (1999) dredged material disposal onto established vegetation in the upland area.

Manitowoc, WI. This CDF is located on the shore of Lake Michigan alongside a marina with associated park facilities, with the top of the inland dike serving as a concrete walkway for pedestrians visiting the marina or park. It is composed of a large ponded area, transitioning into a mud flat, wetland, and upland. The site is still in use for disposal of dredged material, which is pumped over the lake-bordering dike into the CDF. The vegetated portions of the site include from lower to higher elevation low rosette-forming crucifers, *Phragmites australis* (common reed) in the second- or third-year growth stage, and various *Urtica* species (nettles). The site was extensively used by birds, including nesting gulls and geese.

Bayport at Green Bay, WI. This CDF, located on the outskirts of Green Bay on the shore of Lake Michigan, is composed of at least six cells. The sites chosen for sampling are situated within Cell 6. Cell 6 is still in use for the upland disposal of dredged material, and received its last addition in 1999. The dredged material was unstable, and the groundwater table about 5 cm below the dredged material surface. Cell 6 had a uniform elevation, and was completely vegetated by *P. australis* in 2000, but lacked vegetation in 1999.

Dredged Material. At each CDF, three dredged material samples were collected from the soil surface to approximately 0.3-m depth, one in the wettest area (usually close to the point of outflow), one at an intermediate area, and one at the driest area (often the point of entry of the dredged material into the CDF). This sampling strategy was chosen based on the assumption that after entering the CDF, the fine-grained particles in the dredged material are transported toward the point of outflow carrying most contaminants, and the sandy particles settle close to the point of entry. At Monroe, the samples were taken within the recent disposal area following the described sampling strategy. Sampling coordinates for the first sample were 03-06-523 E, 17 46-42-842 N; for the second sample, 03-06-514 E, 17 46-42-869 N; and for the third sample, 03-06-491 E, 17 46-42-853 N. Visual inspection indicated that the dredged material was characterized by a high clay content, and would likely classify as a silty clay or clay loam soil. At Manitowoc, the samples were taken at the edge of the mudflat, halfway between the upland, and within the upland area. Sampling coordinates for the first sample were 4-48-212 E, 16 48-82-813 N; for the second sample, 4-48-213 E, 16 48-82-835 N; and for the third sample, 4-48-188 E, 16 48-22-750 N. Visual inspection indicated that this dredged material was more silty than at Monroe, and would likely classify as a silty loam soil. At Bayport, the samples were taken 15 m away from the dike (i.e., near the weir, halfway, and within the upper end of the cell). Sampling coordinates for the first sample were 4-18-477 E, 16 49-33-556 N; for the second sample, 4-18-444 E, 16 49-33-584 N; and for the third sample, 4-18-392 E, 16 49-33-644 N. Visual inspection indicated that this dredged material was far more organic than at the other locations and somewhat silty.

The dredged material at these three sites has the following characteristics in common that set them apart from most terrestrial soils (Table 1): (1) low organic matter contents (8-15 percent dry weight) compared to most terrestrial soils (this level is high for sediments with organic matter levels on the order of 1 percent), (2) low bulk density (~1 g dry weight (DW) mL^{-1}) typical for structureless soils, (3) relatively high plant-available phosphorus concentration (1.7-2.8 mg kg^{-1}), and (4) relatively low nitrate-nitrogen concentration (0.001-0.012 mg kg^{-1}) and generally high NH_4^+ concentration (not measured in this case). The low nitrate-nitrogen concentration, however, may have been due to the high moisture content of the dredged material and/or relatively long period of 1 week between sample collection and nutrient analyses.

Comparison of the chemical characteristics of dredged materials before and after dredging (Table 1) indicated that differences can be considerable. At Monroe, the concentrations of several metals (cadmium, chromium, nickel, zinc, and copper) were considerably lower at the wettest site (i.e., recently deposited) of the CDF than in the predredged sediment. However, the concentration of mercury was unchanged. Levels of total polychlorinated biphenyls (PCBs) had also decreased, but total polycylic aromatic hydrocarbons (PAHs) were low but unchanged. At Manitowoc, the concentrations of a few metals (cadmium, chromium, and zinc) were considerably lower in the CDF than in the predredged sediment, those of nickel and copper were unchanged, but the levels of arsenic, mercury, iron, and manganese were higher. Levels of total PCBs, PAHs, and pentachlorophenol (PCP) were low and no change was detected. At Bayport, the concentrations of arsenic, cadmium, and chromium were considerably lower in the CDF than in the predredged sediment, nickel was unchanged, but levels of lead, zinc, mercury, copper, iron, and manganese were higher in the CDF. Levels of total PCBs had decreased in the CDF, and those of PAHs and PCP were low and unchanged. Comparison of the chemical characteristics of dredged materials located at different distances from point of entry of the CDF (data not shown) confirmed the assumption that contaminant concentrations increased with distance from the point of entry in the CDF.

No environmental quality benchmarks for dredged material placed in CDFs currently exist. Recent data collected for USEPA's hazardous waste identification rule suggest protective levels for receptor taxa of concern, including terrestrial plants and soil biota (USEPA 1999). Dredged materials in CDFs can be considered as special cases of terrestrial soils, and, therefore, the concentrations listed as protective for terrestrial soil-based communities are likely to be similar to those for CDF dredged materials. Protective levels for terrestrial soils, Chemical Stressor Concentration Levels (CSCL, expressed in mg total element per kg dry soil), vary with the receptor taxa. It was concluded from a comparison of the recent chemical data with the published CSCLs for terrestrial plants and invertebrates (Table 2) that dredged material of the Monroe CDF would be the most suitable substrate to test for effects of metals, since only a few CSCLs for metals and none for organics were exceeded. CSCLs for plants were greatly exceeded for vanadium and zinc; and CSCLs for invertebrates were exceeded by nickel, selenium, and zinc. It was concluded also that none of the dredged material evaluated would be particularly suitable to test for effects of organic contaminants, since the only published CSCL for organics, PCP, was not exceeded for test organisms, and several CSCLs for metals were exceeded.

Table 1
Characteristics of dredged materials collected from three upland CDFs in the Great Lakes area

Characteristic	Confined Disposal Facility					
	Monroe		Manitowoc		Bayport	
	Predredging	CDF Wet Site 06-2000	Predredging	CDF Wet Site 06-2000	Predredging	CDF Wet Site 06-2000
Organics						
PCP		<0.001		0.001		0.003
Total PCB	2.8	1.67	NA	0.07	2.08	1.29
Total PAH		0.02		0.05		0.01
Metals						
Antimony		<2.68		<2.66		<3.24
Arsenic	8.4	8.57	2.55	3.59	7.07	5.35
Beryllium		0.81		0.49		1.07
Cadmium	1.98	1.21	2.6	1.17	6.27	2.106
Chromium (VI)	79.9	<0.54	22.8	<0.53	48.9	<0.65
Lead	51.9	62	44.2	37.5	35.1	87.8
Nickel	45.7	36.18	15.11	18.88	31.9	30.0
Selenium	0.85	<0.54	<1.0	<0.55	ND	<0.63
Silver		<0.67		<0.67		<0.41
Thallium		<0.43		<1.33		<1.62
Vanadium		28.81		28.19		41.79
Zinc	246	201	100.3	89.1		218.7
Mercury	0.29	0.31	0.08	0.19	0.02	1.46
Copper	124	60	28	28	38	86
Iron	21000	24522	14667	22743	25066	29484
Manganese	459	628	436	525	83	771
Aluminum		12515		9735		17658
Nutrients						
Nitrate-nitrogen		0.001		0.002		0.012
Infinite-sink phosphorus*		2.8		1.7		1.8
Total-K		NA		NA		NA
Other						
pH_{water}		7.58		7.54 (6.7**)		7.29 (5.8**)
OM (% DW)		7.83		8.23		15.36
DW (% FW)		65.36		66.35		37.69
BD (g DW/mL)		1.26		1.34		0.88

Note: NA = not analyzed; OM = organic matter; DW = dry weight; FW = fresh weight; BD = bulk density. Concentrations in mg kg^{-1} dry weight.
* Plant-available P-fraction (Van der Zee, Fokkink, and Van Riemsdijk 1987).
** Field measurement.

Table 2
Characteristics of dredged materials and soil types included in the plant and invertebrate tests

Characteristic	Concentration, mg kg^{-1} dry weight				
	Monroe		Plant Control		Invertebrate Control Total
	Total	DTPA-Extractable	Total	DTPA-Extractable	
Organics					
PCP	<1.5		<1.7		
Total PCB	1.788		1.07		
Total PAH	6.83		3.21		
Metals					
Antimony	<0.1		<0.1		
Arsenic	12.06		8.08		2
Barium	NA		NA		
Beryllium	1.07		0.59		
Cadmium	2.04		2.28		0.29
Chromium (VI)	<0.04 (VI)		<0.04 (VI)		7.2 (tot)
Lead	70.88	0.41±0.02	44.5	0.74±0.01	21.8
Nickel	57.21	0.43±0.1	27.25	0.12±0.01	6.8
Selenium	1.87		1.42		
Silver	0.4		0.35		0.1
Thallium	0.8		0.35		
Vanadium	53.19	0.09±0.00	28.91	<0.05	
Zinc	273.36	5.04±0.33	135.66	4.51±0.23	55.6
Mercury	0.54		0.24		0.14
Copper	73.7		36.05		14.8
Iron	39530		19397		
Manganese	904.5		401.03		
Aluminum	23048		14756		
Nutrients					
Nitrate-nitrogen	19.4±0.5		122.6±26.1		NA
Infinite-sink phosphorus	3.62±0.91		14.03±9.79		NA
Total-K	NA		NA		1090
Other					
pH_{water}	7.25		5.79		7.0-7.5
OM (% DW)	10.5		76.29		NA
DW (% FW)	49.5		41.8		NA
BD (g DW/mL)	7.9		9.3		NA

(Continued)

Note: CSCLs (USEPA 1999) are given for comparison, and levels in the crust of the earth are given for reference. All determinations were performed on fresh dredged materials and soils.
DTPA = diethylamine-pentaacetic acid.

Table 2 (Concluded)

Characteristic	Concentration, mg kg^{-1} dry weight		
	USEPA Guideline		Earth Crust Level
	CSCL Plants	CSCL Inverts	
Organics			
PCB	3	6	
Total PCB			
Total PAH			
Metals			
Antimony	5		1
Arsenic	10	60	5
Barium	500		430
Beryllium	10		6
Cadmium	4	1	0.2
Chromium (VI)	1 (VI)	0.4 (VI)	200 (tot)
Lead	50	28	10
Nickel	30	90	100
Selenium	1	70	0.09
Silver	2		0.02
Thallium	1		12
Vanadium	2		150
Zinc	50	100	80
Mercury	0.1 (2+)		0.5
Copper			70
Iron			50000
Manganese			1000
Aluminum			81000
Nutrients			
Nitrate-nitrogen			
Infinite-sink phosphorus			
Total-K			26000
Other			
pH_{water}			
OM (% DW)			
DW (% FW)			
BD (g DW/mL)			

Macroinvertebrates. At each CDF, three surface litter samples, approximately 2 cm deep in an area of 930 cm^2, were collected concurrent with the dredged material samples. First large macroinvertebrates, such as earthworms and large arthropods, were removed from the samples within 4 days after collection, and stored in 95 percent isopropanol until genera were determined. Subsequently, a portion of the dredged material was placed in a Berlese funnel apparatus (15-cm funnel diameter) for extraction of the smaller invertebrates (Borror and DeLong 1964). The extraction procedure was allowed to proceed for 14 days to provide sufficient time for animals to move through the dredged material column and into the alcohol. Identifications were made to the taxa levels, with nomenclature following Chu (1949), Kaston and Kaston (1953), Borradaile et al. (1961), and Borror and DeLong (1964).

The Monroe CDF was inhabited by relatively few invertebrates (Table 3) with small population sizes. Soft-bodied invertebrates, such as gastropods and annelids, were absent. Most of the groups identified were insects, foraging on the surface (beetles, flies including larvae, beetle larvae, spiders) and living in the dredged material (collembola or springtails). Progressing upland along a transect to the drier sites, the taxa remained the same but numbers of individuals per sample were smaller.

At the wet Manitowoc site a large and diverse Collembola (springtail) population occurred, but few members of other taxa that were found at relatively higher diversities (at the class level) at both drier Manitowoc sites. At the driest Manitowoc site, two earthworm species, centipedes, and millipedes were identified. Dredged material sampled at the Manitowoc CDF had remained undisturbed for a long time (several years) relative to that at the Monroe and Bayport Cell 6 sites. Both driest Manitowoc locations, which were covered with herbaceous vegetation, supported large and diverse invertebrate populations of earthworms, isopods, chilopods, diplopods, and insects. The animals present, especially the large number of isopods, tend to illustrate the typical "boom and bust" population dynamics of highly disturbed ecosystems. Species composition and population density of the major taxa may be expected to fluctuate from year to year with the vegetation.

At the Bayport sites, soil-dwelling invertebrates were very sparse, with only a few surface foraging animals. This may have been because the dredged material was recently placed in the site, the site was wet and unconsolidated, and time was insufficient to allow normal soil processes to develop and provide the microhabitats necessary for colonizing soil invertebrates.

Summarizing, invertebrate diversity and abundance varied per site, both being highest at sites where the dredged material had become consolidated, the water table had decreased, and vegetation had been established providing organic matter and microhabitats required for colonization by the animals. In general, the time elapsed since the additions of dredged material to the CDFs has been insufficient for the development of a stable vegetation cover, and the organic material and root structure of the plants have been insufficient for the development of soil depth profiles. As a consequence, the dredged materials in these CDFs is depauperate in soil macrofauna and meiofauna. Among the taxa identified, springtails were present at six out of nine sites, and earthworms at one out of nine sites. Based on their relevance for these toxicity tests, springtails and earthworms would be suitable test organisms.

INITIAL TOXICOLOGIAL TESTING: Based on the recent chemical information on the dredged material of the three CDFs, the Monroe CDF dredged material was selected for toxicological testing

Table 3
Characteristics of macroinvertebrates collected from three upland CDFs in the Great Lakes area

Location	Taxa Collected		Comments
Monroe CDF			
Wet site	Insecta	Collembola	
		Coleoptera	Larvae and adults
		Diptera	Larvae and adults
	Arachnida	Araneae	
Intermediate site	Insecta	Collembola	
		Diptera	Adults
	Arachnida	Araneae	
Dry site	Insecta	Collembola	
		Coleoptera	Larvae only
		Diptera	Larvae and adults
	Arachnida	Araneae	
Manitowoc CDF			
Wet site	Insecta	Collembola	Five species, large population
		Coleoptera	
		Diptera	Larvae and adults
	Arachnida	Araneae	
	Gastropoda		Snail
Intermediate site	Insecta	Collembola	
		Coleoptera	Adults
		Diptera	Adults
	Arachnida	Araneae	
	Isopoda		Large population
Dry site	Oligochaeta		Two earthworm species
	Insecta	Collembola	
		Coleoptera	Larvae
		Diptera	Adult
	Arachnida	Araneae	
	Isopoda		Large population
	Chilopoda		
	Diplopoda		Snail
	Gastropoda		
Bayport Cell 6			
Wet site			No animals found
Intermediate site	Arachnida	Araneae	
	Gastropoda		One slug
Dry site			No animals found

in the expectation that potential toxic effects could be attributed to the presence of inorganics in the dredged material. As bioassay organisms, common species that might occur at disturbed sites were used, although they were not identified in the recent survey.

Dredged material was collected at the wettest site of the Monroe CDF. It was transported from Michigan to the Environmental Laboratory, Vicksburg, MS, U.S. Army Engineer Research and

Development Center, in drums, shipped in cooled overpacks. Upon arrival, the dredged material was dried so that the moisture content was reduced to about 50 percent and the innate microbial community could persist. Treatment included spreading and mixing the dredged material on plastic sheets inside a greenhouse and turning it until it contained approximately 50 percent (by weight) water. As a control substrate, two organic soils were chosen. Baccto R Lite potting soil (85-90 percent organic peatmoss), a highly organic, nutritious soil, Michigan Peat Company, Houston, TX, was used for plants. For invertebrates, another highly organic soil was purchased from Carolina Biological Supply Company, Burlington, NC. The test and both control soils were chemically and physically characterized prior to testing (Table 2). The control soil for plants was nutritious (six times more nitrogen and phosphorus than the test dredged material) and generally contained far lower contaminant levels. However, it contained total vanadium and zinc levels that exceeded the proposed plant CSCLs. The control soil for the invertebrates, on the other hand, contained a relatively high total chromium concentration, that probably exceeded the proposed invertebrate CSCL (CrVI is estimated to be about 10 percent of total chromium). A reference soil, in the sense of an uncontaminated soil of similar composition to that of the test soil, was not included in the plant and invertebrate tests.

PLANT TESTS

Experimental Design, Plant Material, Culture Conditions, and Analyses. A multifactorial experimental design using a completely randomized design with four replicates per treatment was used with the following treatments: (1) substrate type, (2) plant species, (3) pot size, and (4) test duration. Two plant species were included, *Cyperus esculentus* (yellow nutsedge), a plant species commonly used at the Environmental Laboratory as an index plant, and *Cynodon dactylon* (bermudagrass). The latter species was selected for its wide geographical distribution, being common in southern regions of North America, and rapid growth and profuse generative reproduction; and because most of its biomass is aboveground. The latter characteristic would facilitate harvesting, and reduce sample number and analytical costs by a factor of two. Two pot types with the same 2-L volume were tested, small pots with a 0.0167-m^2 surface area and large pots with a 0.0238-m^2 surface area. Three test durations were tested: for *C. esculentus* 21, 35, and 63 days; and for *C. dactylon* 21, 63, and 77 days.

The experiment was started on 7 July 2000, with four pregerminated tubers of *C. esculentus* per pot and 0.207 g *C.dactylon* seeds per 0.028-m^2 pot surface, in a greenhouse at the Environmental Laboratory. The plant propagules were purchased as follows: *C. esculentus* as tubers from Wildlife Nurseries, Inc., Oshkosh, WI, and *C. dactylon* as common *C. dactylon* seeds from the Flower Center, Vicksburg, MS. Pots were placed in saucers, with saucers daily replenished with demineralized water. Test and control soils were kept at a soil moisture level of approximately 50 percent. Soil moisture levels were monitored using irrometers (Irrometer Company, Riverside, CA). At harvest time, each species was removed from its pot, *C. esculentus* was divided into aboveground and belowground organs, and the plant material was manually freed from soil particles, rinsed with demineralized water, blotted dry, weighed, and dried in a forced-air oven to constant weight (70 °C). Growth response was expressed in g dry weight per m^2. Metal concentrations were determined in the plant material of the second and third harvests by acid-digesting 0.5 g dry plant material in a microwave-oven for lead, vanadium, nickel, and zinc followed by measuring total

metals using Inductively Coupled Plasma Analysis (ICP) (USEPA Method 3050 (USEPA 1996)), or cold-vapor determination for mercury (USEPA Method 7471A (USEPA 1994)).

Toxicity. Analysis of variance (ANOVA) of the complete data set indicated that the effect of substrate on growth response (i.e., total biomass) was not statistically significant, but the effects of plant species and test duration were significant ($P < 0.001$). The data are graphically represented in Figures 2 and 3. This means that the differences in growth responses of both plant species could not be attributed to differences in substrate type alone, i.e., test dredged material being phytotoxic compared to control soil, but that they depended on plant species and on test duration. The effect of pot size on the belowground plant biomass of *C. esculentus* was significant, with more biomass being produced in the relatively deeper pots with the smallest surface area ($P < 0.05$). The effect of pot size on total biomass of *C. dactylon* (roots were so small that belowground and aboveground biomass was not distinguishable) was also significant, with more biomass being produced in the pots with the largest surface area ($P < 0.05$).

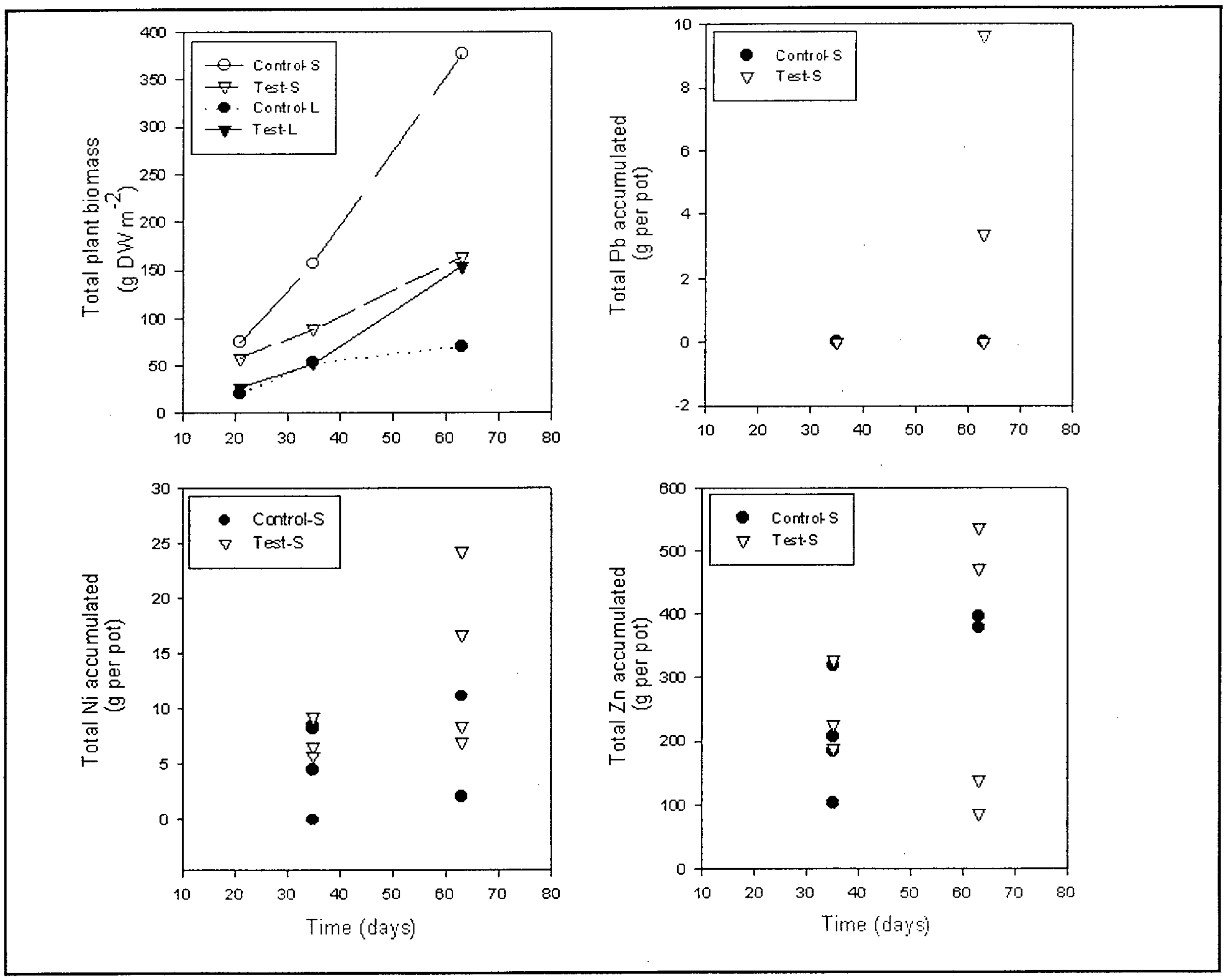

Figure 2. Average growth response (N = 4) of *C. esculentus* plants cultivated on test dredged material and control soil in 2-L pots, with a small (S, 0.0167 m^2) and large (L, 0.0238 m^2) surface area (top left). Bioaccumulation of lead, nickel, and zinc was also measured; of the bioaccumulation data, only those pertaining to pots with a small surface area are shown

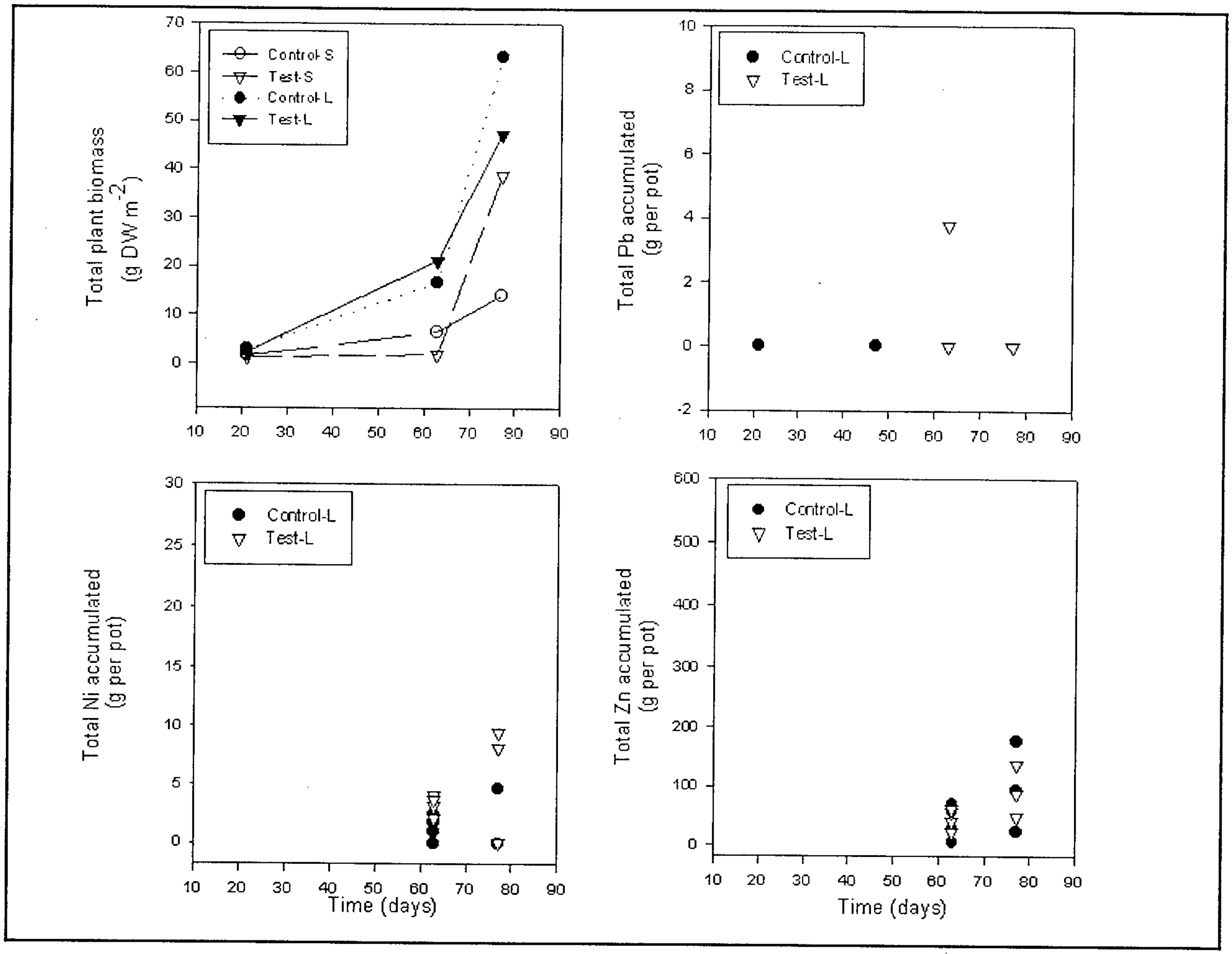

Figure 3. Average growth response (N = 4) of *C.dactylon* plants cultivated on test dredged material and control soil in 2-L pots, with a small (S, 0.0167 m^2) and large (L, 0.0238 m^2) surface area (top left). Bioaccumulation of lead, nickel, and zinc was also measured; of the bioaccumulation data, only those pertaining to pots with a large surface area are shown

Bioaccumulation. Plant species exhibited a different metal-specific behavior with respect to bioaccumulation. *C.esculentus* accumulated lead from 63 days onward in the belowground biomass (3.31 $\pm$ 3.96 mg kg^{-1}); nickel in 35 days 5.07 $\pm$ 2.38 mg kg^{-1} in aboveground biomass and in 63 days 12.2 $\pm$ 5.27 mg kg^{-1} in belowground biomass; zinc from day 35 onward (176 $\pm$ 7.96 mg kg^{-1} in aboveground and 136 $\pm$ 21.3 mg kg^{-1} in belowground biomass). *C.dactylon* initially accumulated lead, reaching a level of 1.50 $\pm$ 2.99 mg kg^{-1} at 35 days and subsequently depurating it to an undetectable level. It accumulated nickel from 35 days onward up to 6.89 $\pm$ 1.29 mg kg^{-1}; and zinc to 93.6 $\pm$ 6.52 mg kg^{-1}. None of the species accumulated vanadium or mercury. It has to be noted that nickel and zinc accumulation in plants cultivated on the control soil was substantial compared with that of plants cultivated on test soil .The total amount of metals accumulated in plant biomass was usually higher in *C. esculentus* than in *C. dactylon*, with a large part stored belowground in the former species.

Summary Plant Test Results. Both plant species may be suitable test organisms for the inorganic chemicals of concern (COCs) in dredged material in that they survived on dredged

material and produced enough biomass in about 2 months to allow evaluation of (1) a growth response, and (2) the bioaccumulation of metals. The lack of profound plant toxicity was consistent with the low levels of contaminants in the dredged material. However, the relatively large variability may have masked more subtle toxic effects. Bioaccumulation characteristics depended on both plant and metal species. More work needs to be done to standardize plant toxicity testing (e.g., pot shape). Most of the variability was attributed to the nonsynchronous germination of tubers and seeds. It is to be expected that synchronization of growth, e.g., by artificial breakage of dormancy, will yield less variability in the test results. Such a treatment may also allow for a shorter duration of the test period, required for the production of sufficient biomass for the various analyses (currently 63 days). It may also enable the plants to reach their reproductive stage. In the current experiment only one *C. dactylon* plant flowered. The lower growth response on the test dredged material does not necessarily point in the direction of dredged material toxicity; it may be due partly to a more severe limitation by the low nitrogen supply compared with that of the control soil (Table 2). It is recommended that for future testing, test and reference substrate are fertilized with nitrogen and phosphorus to levels considered sufficient to support growth of the test species. For cases where turf grass species common in pastures are used, fertilization levels in the order of 352 kg nitrogen ha^{-1} $year^{-1}$, 59.2 kg phosphorus ha^{-1} $year^{-1}$, and 331.9 kg potassium ha^{-1} $year^{-1}$, supplied in mineral + organic form, are recommended in Western Europe (Best and Jacobs 2001). The 2-L pot size used in the current experiment allowed the production of sufficient biomass for metal analysis, i.e., $\geq$ 0.5 g DW per pot. Relatively deeper pots were more suitable for *C. esculentus*, and pots with a relatively large surface area more suitable for *C. dactylon*. Statistical exploration of the data set indicated that each test, composed by four dilutions of the test dredged material (including the reference) with only one harvest date, should include $\geq$5 replicates to generate statistically significant results.

Use of Bioaccumulation Data in the Plant Uptake Program (PUP). Since 1990, a computerized procedure for predicting plant uptake of heavy metals from contaminated freshwater dredged material has been used at the Environmental Laboratory. This system is composed of a database that relates the shoot concentrations of arsenic, cadmium, chromium, lead, nickel, zinc, mercury, copper, iron, and manganese in *C. esculentus*, and of cadmium, lead, zinc, and copper in various agronomic plants, to the DTPA- (diethylamine-pentaacetic acid) extractable metal concentrations in the substrate on which the plants were cultivated via linear regression. Effects of pH and organic matter content of the substrate on metal bioavailability are taken into consideration by the procedure. The DTPA-extractable metal fraction is considered to be a fair measure for the bioavailable fraction of metals (Lindsay and Norvell 1978). The latter fraction can measure up to 5 percent of the total metal concentration. The user is required to enter the following data into the program: the DTPA-extractable metal concentrations, pH, and organic matter content of (1) upland or air-dried test dredged material; (2) flooded or wet test dredged material; and (3) upland or air-dried reference dredged material. The procedure provides the user subsequently with (1) the shoot metal concentrations expected to be exceeded in plants cultivated on the test soil compared with that of plants cultivated on the reference soil, and (2) the actual shoot concentrations expected.

For the Monroe CDF case, the test dredged material data were entered as representing upland or air-dried dredged material, the control soil data were entered as representing upland or air-dried reference dredged material; no data were entered for flooded or wet test dredged material. The DTPA-extractable fractions of lead, nickel, vanadium and zinc (mercury was not determined) were very low in the Monroe test dredged material, i.e., maximally 1 percent (Table 2). Vanadium data

were not entered because the program's database did not contain information on vanadium. The PUP procedure predicted shoot metal concentrations that were outside the currently measured range. Discrepancies were attributed to the use of a highly organic soil instead of a reference soil with the same characteristics as the test soil (Folsom, Davis, and Houghton 1988; Folsom and Price 1989; Folsom and Houck 1990), and to the procedure's assumed linear relationship between the total metal concentration in the soil and in the plant shoots.

INVERTEBRATE TESTS:

Experimental Design, Animal Material, Culture Conditions, and Analyses. Three separate experiments were conducted: two using the lumbricid compost worm *Eisenia fetida* and one using the enchytraeid worm *Enchytraeus crypticus* as a test organism. *E. fetida* is the standard test organism used in terrestrial ecotoxicology and is widely used in North America and Western Europe. The latter worm has an epigean, litter-dwelling life strategy, and reproduces via cocoons. *E. crypticus* is an organism whose potential use as a bioassay organism for ecotoxicological testing within North America and Western Europe is being explored. It is believed to feed on decomposing plant material and associated microorganisms. The animals were surface-fed with the same food as used for *E. fetida*. Toxicological effects in these test species stem largely from direct skin contact with the toxic compounds in the interstitial water (Lokke and Van Gestel 1998).

Experiment I. A bifactorial experiment, using a completely randomized design with four replicates per treatment, was conducted with the following treatments: (1) substrate type, and (2) test duration. Four durations were tested, 12, 28, 42, and 56 days. The experiment was started on 14 July 2000, with 20 adult *E. fetida* specimens per replicate cylinder (15-cm-diameter, 15-cm-high Plexiglas; 1,040 g soil fresh weight per cylinder), and four replicates per treatment. Testing occurred at 20 °C under continuous fluorescent illumination, as described in American Society for Testing and Materials (ASTM) (1998). *E. fetida* specimens were taken from the Environmental Laboratory culture, originally purchased from Carolina Biological Supply Company, Burlington, NC. Food was supplied regularly as needed. It was composed of rolled oats, purchased locally, and powered earthworm food from Magic Products, Inc., Amherst Junction, WI. Each harvest time, $\geq$12 specimens were removed from their cylinders, weighed, and deep-frozen until further processing. Subsequently, the animals were dried in an oven to constant weight (24 hr at 70 °C). Growth response was expressed in g dry weight per 20 individuals, and reproductive potential as number of cocoons per cylinder. Metal concentrations were determined in all samples by digesting 0.5 g dry animal material in a microwave oven for lead, vanadium, nickel, and zinc (USEPA Method 3050 (USEPA 1996)), or cold-vapor determination for mercury (USEPA Method 7471A (USEPA 1994)).

Experiment II. This experiment was similar to the former one, except that it was started with 12 juvenile *E. fetida* specimens to explore the youngest possible age at which toxic effects became apparent. It lasted 65 days. Animal biomass and reproductive potential were determined, but bioaccumulation was not measured.

Experiment III. This experiment was performed to explore culture conditions and culture duration required to harvest sufficient biomass for analysis using a test organism new to the Environmental Laboratory. Plastic petri dishes were filled with a preweighed quantity of substrate to be tested, and

inoculated with 8-10 *E. crypticus* specimens from a mass culture, using a dissecting needle. Mass cultures were obtained from R. Kupperman (U.S. Army Aberdeen Proving Ground, MD). Substrates tested were (1) Monroe CDF dredged material, (2) invertebrate control soil, and (3) standard Organization for Economic Cooperation and Development artificial soil (10 percent finely ground peat moss, 20 percent kaoline clay, 70 percent silica sand, on an oven-dry weight basis (ASTM 1998). All petri dishes were moistened regularly with demineralized water and the animals were fed and incubated at 16 °C for 21 days. All treatments generated live worms at the end of the incubation period, but counting of individual worms and separation of worms and substrate proved extremely time-consuming. It is, therefore, concluded that prior to further testing a culture method has to be selected that allows better separation of substrate and test organism.

Toxicity.

Experiment I. ANOVA of the complete data set indicated that the effect of substrate on adult biomass was not statistically significant, but its effect on reproductive potential was significant ($P < 0.001$). The effect of test duration was significant on both adult biomass and reproductive potential. This indicates that reproductive potential is a more sensitive parameter for toxicological effects than adult biomass (as found also by Robidoux et al. 2000), and that the test results depend on test duration. The data are graphically represented in Figure 4.

Experiment II. The results of this experiment largely confirmed those of Experiment I, but the variability in growth response was lower (data not shown).

Bioaccumulation.

Experiment I. Bioaccumulation of zinc occurred in animals cultivated on test dredged material as well as on control soil, and effects of substrate on the animal zinc concentrations were not significant. Bioaccumulation of mercury became apparent after 42 days, and the effect of substrate on animal mercury concentration was significant (Figure 4).

Summary Invertebrate Test Results. *E. fetida* is a suitable test organism for the inorganic COCs in dredged material, in that the adult specimens survived on dredged material and produced enough biomass and cocoons in about one month to allow the evaluation of a growth response, reproductive potential, and the bioaccumulation of several metals, i.e., zinc and mercury. Reproductive potential proved to be a more sensitive parameter for toxicity than biomass. As discussed earlier for the plant tests, the relatively large variability may have masked more subtle toxic effects. Variability was substantially less in the experiment, started with juvenile worms. It is to be expected that synchronization of growth will yield less variability in the test results. A test duration of 28 days started with 20 adult specimens in 1-kg cylinders proved long enough to allow sufficient biomass formation for metal analysis, i.e., ≥ 0.5 g DW per cylinder. Statistical exploration of the data set indicated that each test, composed by four dilutions of the test dredged material, including the reference, with only one harvest date, should include ≥ 5 replicates to generate statistically significant results. A potential drawback affecting the use of *E. fetida* is that the organism requires the presence of a relatively well-developed plant litter layer for its persistence. *E. crypticus* requires a less organic habitat, and could serve as a test organism for dredged materials with very low organic

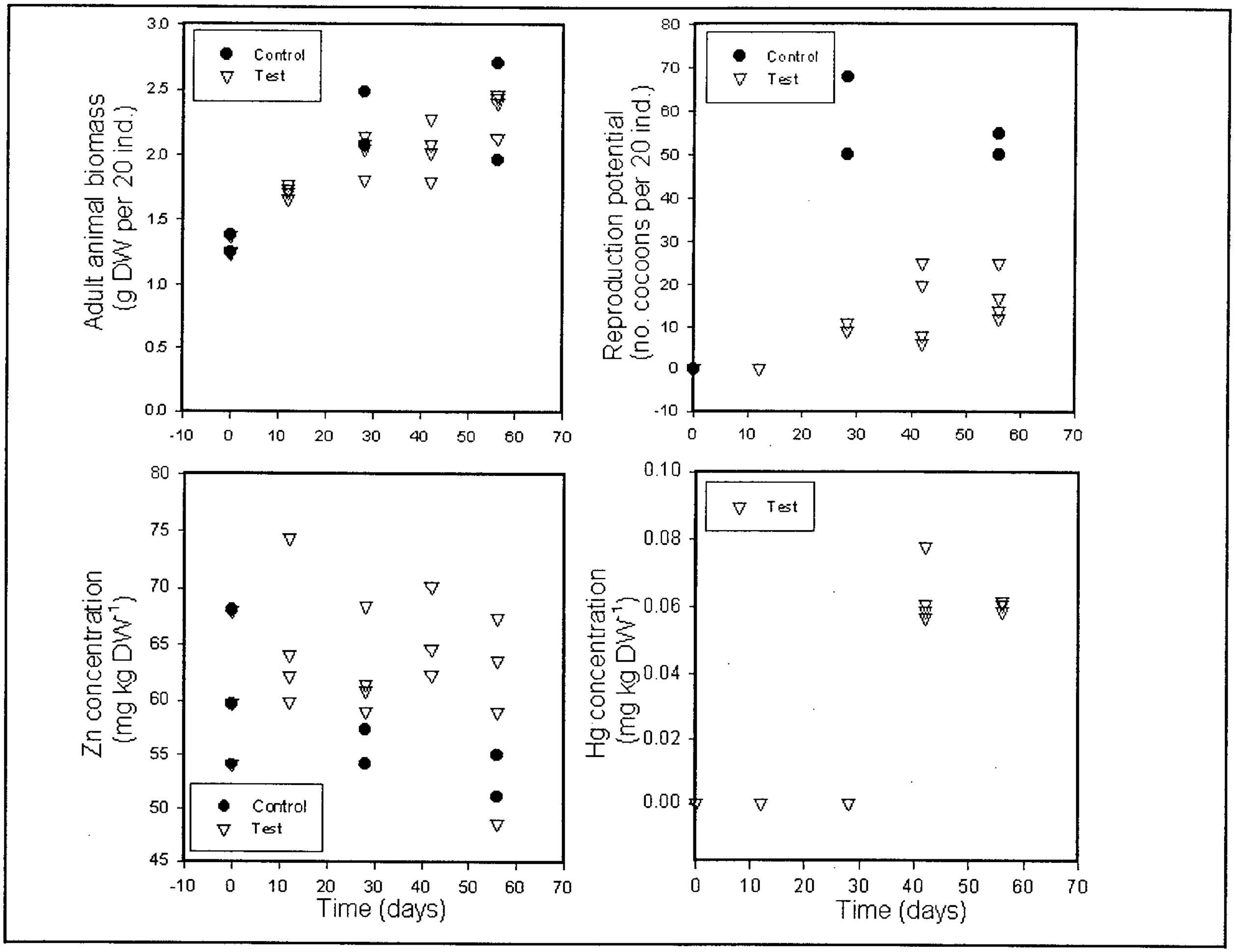

Figure 4. Growth and reproductive response, and bioaccumulation of zinc and mercury in adult *E. fetida* worms cultivated on test dredged material and control soil in 1-kg cylinders

matter contents. However, at this time use of the enchytraeid worm, *E. crypticus*, for dredged material toxicity screening needs additional development and testing.

SUMMARY: Three CDFs in the Great Lakes area with known predredging contaminant types and levels were initially selected for verification of dredged material chemical composition, evaluation of nutrient levels, and preliminary survey of landscape and biota: Monroe, MI, Manitowoc, WI, and Bayport, WI. Typical characteristics of the dredged materials that set them apart from most soils are the low organic matter content (compared to soils), low bulk density, relatively high plant-available phosphorus, and low nitrate-nitrogen concentrations. All sites are characterized by the presence of a ponded area, an intermediate area occupied by herbaceous vegetation typical for highly disturbed systems, and a forested upland area. Invertebrate diversity and abundance varied per site, both being highest at sites where the dredged material had become consolidated, the water table had decreased, and vegetation had been established. Springtails and earthworms were fairly common, and are considered as suitable test organisms for these dredged materials. Dredged material of the Monroe site was selected for further bioassay testing of inorganic contaminants. Suitable plant species for testing were found to be *Cyperus esculentus*, yellow nutsedge, and *Cynodon dactylon*, common bermudagrass. *Eisenia fetida*, an earthworm, was identified as a

suitable invertebrate test species, with *Enchytraeus crypticus* as a potential second candidate, the latter particularly suitable for dredged materials with low organic matter contents. Of these four species *C. esculentus* and *E. fetida* have been previously used as test species at the Environmental Laboratory. Test protocols were further developed and streamlined, and collected data of several fact-finding experiments evaluated. The experience will be used to perform and evaluate exposure-based effects assessments of contaminants in terrestrial plants and animals, and to develop guidance to standardize the interpretation of test results in risk assessment aimed at determining effective long-term management strategies for CDFs.

POINTS OF CONTACT: For additional information, contact Dr. Elly P. H. Best (601-634-4246, *Elly.P.Best@erdc.usace.army.mil*) or the Program Manager of the Dredging Operations and Environmental Research Program, Dr. Robert M. Engler (601-634-3624, *Robert.M.Engler@erdc.usace.army.mil*). This technical note should be cited as follows:

> Best, E. P. H., Tatem, H. E., and Winfield, L. (2001). "Toxicological and bioaccumulation testing of dredged material in confined diposal facilities using plants and worms," *DOER Technical Notes Collection* (ERDC TN-DOER-C22), U.S. Army Engineer Research and Development Center, Vicksburg, MS.
> *www.wes.army.mil/el/dots/doer*

REFERENCES

American Society for Testing and Materials. (1998). "Standard guide for conducting laboratory soil toxicity or bioaccumulation tests with the lumbricid earthworm *Eisenia fetida*," E 1676-97, Annual Book of ASTM Standards, Vol. 11.05, West Conshokocken, PA, 1056-1074.

Best, E.P.H., and Jacobs, F. H. H. (2001). "Production, nutrient availability, and elemental balances of two meadows affected by different fertilization and water table regimes" (in press), *Plant Ecol.*

Borradaile, L. A, Potts, F. A., Eastham, L. E. S., and Saunders, J. T. (1961). *The Invertebrata.* Cambridge University Press, Cambridge, UK.

Borror, D. J. , and DeLong, D. M. (1964). *An Introduction to the study of insects.* Holt, Rinehart, and Winston, New York.

Chu, H. F. (1949). *How to know the immature insects.* William C. Brown, Dubuque, IA.

Folsom, B. L., and Houck, M. H. (1990). "A computerized procedure for predicting plant uptake of heavy metals from contaminated freshwater dredged material," *Environmental Effects of Dredging Technical Notes Collection* (TN EEDP-04-12), U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Folsom, B. L., and Price, R. A.(1989). "A plant bioassay for assessing plant uptake of heavy metals from contaminated freshwater dredged material," *Environmental Effects of Dredging Technical Notes Collection* (TN EEDP-04-11), U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Folsom, B. L., Davis, B. E., and Houghton, N. J. (1988). "Heavy metal uptake by agronomic crops and *Cyperus esculentus* grown on oxidized and reduced soils contaminated with metal-mining wastes," *Environmental Effects of Dredging Technical Notes Collection* (TN EEDP-02-6), U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Kaston, B. J., and Kaston, E. (1953). *How to know the spiders.* William C. Brown, Dubuque, IA.

Lindsay, W. L., and Norvell, W. A. (1978). "Development of a DTPA soil test for zinc, iron, manganese and copper," *Soil Sci. Soc. Am. J.* 42, 421-428.

Lokke, H., and Van Gestel, C. A. M. (1998). "Soil toxicity tests in risk assessment of new and existing chemicals." *Handbook of soil invertebrate toxicity tests*. Chapter 1, H. Lokke and C. A. M. Van Gestel, ed., John Wiley, New York.

Moore, D. W., Bridges, T. S., and Cura, J. (1998). "Use of risk assessment in dredging and dredged material management," *DOER Technical Notes Collection* (TN DOER-R1), U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS. *www.wes.army.mil/el/dots/doer/*

Robidoux, P. Y., Svendsen, C., Caumartin, J., Hawari, J., Ampleman, G., Thiboutot, S., Weeks, J. M., and Sunahara, G. I. (2000). "Chronic toxicity of energetic compounds in soil determined using the earthworm (*Eisenia andrei*) reproduction test," *Environ. Toxicol. Chem.* 19, 1764-1773.

Rundgren, S., and Augustsson, A. K. (1998). "Test on the Enchytraeid Cognettia sphagnetorum (Vejdovsky) 1877," *Handbook of soil invertebrate toxicity tests*. Chapter 6, H. Lokke and C. A. M. Van Gestel, ed., John Wiley, New York.

U.S. Environmental Protection Agency. (1994). "Test methods for evaluating solid wastes, Physical/chemical methods, Final update III, Method 7471a," SW-846, 3rd ed., Office of Solid Waste and Emergency Response, Washington, DC.

U.S. Environmental Protection Agency. (1996). "Test methods for evaluating solid wastes, Physical/chemical methods, Final update III, Method 3050b," SW-846, 3rd ed., Office of Solid Waste and Emergency Response, Washington, DC.

U.S. Environmental Protection Agency, Office of Solid Waste. (1999). "Data collection for the hazardous waste identification rule. Section 14.0 Ecological benchmarks," prepared by Center for Environmental Analysis, Research Triangle Institute, Research Triangle Park, NC, under Contract No. 68-W-98-085, Washington, DC.

U.S. Environmental Protection Agency and U.S. Army Corps of Engineers (1991). "Evaluation of dredged material proposed for ocean disposal: Testing manual," EPA-503/8-91/001, Washington, DC.

U.S. Environmental Protection Agency and U.S. Army Corps of Engineers (1992). "Evaluating environmental effects of dredged material alternatives – A technical framework," EPA-842-B-92-008, Washington, DC.

U.S. Environmental Protection Agency and U.S. Army Corps of Engineers (1998). "Evaluation of dredged material proposed for discharge in waters of the U.S.- Testing manual," EPA-823-B-98-004, Washington, DC.

Van der Zee, S. E. A. T. M., Fokkink, L. G. J., and Van Riemsdijk, W. H. (1987). "A new technique for assessment of reversibly adsorbed phosphate," *Soil Sci. Soc. Amer. J.* 51, 599-604.

NOTE: The contents of this technical note are not to be used for advertising, publication, or promotional purposes. Citation of trade names does not constitute an official endorsement or approval of the use of such products.